 Printed in U.S.A.

EFFECTS OF THE PINEAL GLAND UPON THE HAIR CYCLES IN MICE

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Hair growth in rats and mice is cyclic, each hair follicle having alternate periods of growth and rest. Also, the hair follicles develop in waves of hair growth which cover gradually the skin with a new coat of hair (1, 2, 3, 4, 5).

Diverse endocrine factors may be stimulants or inhibitors, modifying the initiation, advance and characteristics of the hair wave (6, 7, 8, 9, 10). The inhibitory hormonal factors appear to be of pituitary origin, and its effect on the adrenals and gonads (6, 9, 10, 11). The stimulating hormonal factors are in part hypophyseal and in part extrahypophyseal (10, 11). Part of the stimulating action of the pituitary is produced by means of the thyroid (7, 8, 12, 13).

The nervous system also intervenes in the regulation of the hair cycle. Slow hair waves were found in hemiarquidecorticated rats. This inhibitory effect is that of gonad stimulation, as shown by its suppression through castration (14).

Lesions of the olfactory cortex, especially the anterior and internal part of the tuberculum olfactorium, resulted in slower pace of advance of hair waves both in normal and in castrated mice (15).

These data suggested the hypothesis of the neurohormonal control of the hair growth waves in rats and mice. Thus, mechanisms probably located in the nervous system, would regularly alter the balance between the stimulating and inhibitory factors (15).

The pineal gland is considered to be a neuroendocrine transducer and to regulate ovarian function in mammals (16). Melatonin, active principle of the pineal gland, lightens the amphibian skin through its effect upon melanocytes (17). Thus, the pineal gland has an effect both on the skin of amphibia and on some endocrine glands in mammals (16, 17, 18). The experiments presented in this paper were performed to ascertain its assumed role on the initiation and on the advance of hair growth waves in mice.

MATERIAL AND METHODS

Male C_3 H/Ep mice, approximately 3 months old, from the Animal Colony of the Comisión Nacional de la Energía Atómica, Argentina, were used. The animals were fed on pellets of a common laboratory diet (Forramez Lab, Molinos del Río de la Plata, Buenos Aires, Argentina). Water was given *ad libitum*.

Pinealectomy was performed in some groups of animals using the technic of Pazo. A hole was made with a dental burr in the right parietal bone, near the back border, close to the midline. A 15 gauge needle was inserted through the hole and the pineal gland was removed by aspiration with a vacuum pump. The completeness of the operation was checked when autopsy was performed. The control animals were sham-operated, following the same procedure except that the suction was not applied and the pineal gland was not removed.

Radiothyroidectomy was performed with 100 microcuries of I 131 in some groups of animals, 60 days before the pinealectomy or the sham operation. Castration was performed in some groups of thyroidectomized mice the day following the pinealectomy or the sham operation.

Melatonin was generously provided by Prof. A. Lerner, Yale University. It was dissolved in absolute alcohol as to make a 5% stock solution. This material was added to saline for the injections. A daily dose of 100 micrograms was injected subcutaneously during 56 days.

Every animal was clipped 7 days before pinealectomy or the sham operation with an electric clipper number 0000, over an area extending along the back, from the neck to the base of the tail.

The clipped area was observed twice a week throughout the experiment and the percentage of denuded area covered by hair was estimated once a week using the following method. The contours of the denuded surfaces were drawn in a transparent paper of uniform width. The contours were then cut out and these areas weighed in a Mettler H 5 balance. The weight of the cuts, when the animal was clipped (0 day cuts), was compared with the other measurements in the same animal using the following formula:

This investigation was partially supported by the research grant N° 499 A on the "Neurohormonal regulation of hair growth waves in rats and mice" from the Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina.

Received for publication November 27, 1965.

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Hair growth in denuded area (%)

$$= 100 - \frac{\text{Weight of the cut} \times 100}{\text{Weight of the 0 day cut}}$$

At the end of the experiments the animals were killed with ether, and the adrenals, pituitary, thyroid and testicles were carefully dissected and weighed.

The mean and standard error were calculated in every group of data and the probability was found using the "t" index of Fischer and Yates.

RESULTS

Effects of pinealectomy in normal mice.—In Experiment 1, the hair growth in the clipped areas was observed in 8 pinealectomized mice, compared with 9 sham-operated controls. As it is shown in Figure 1, a larger area was covered by hair in the pinealectomized mice than in the controls at the different intervals observed. After 53 days all the pinealectomized mice and none of the sham-operated controls had the clipped area completely covered by hair. The average area covered with hair in the sham-operated mice was 80.7%.

The experiment was repeated several times and the clipped area was always covered with hair more rapidly in the pinealectomized mice than in their sham-operated controls.

The animals in Experiment 1 were killed 53 days after pinealectomy or the sham operation, the weight of the organs being presented in Table I. There was a significant hypertrophy of the thyroid ($p < 0.001$) and the testicles ($p < 0.01$). No significant changes were found in the weight of pituitary and adrenals.

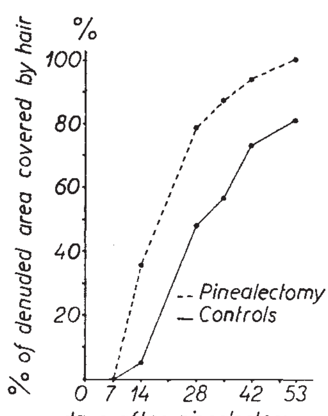

FIG. 1. Hair growth in pinealectomized and sham-operated mice.

TABLE I

Effects of Pinealectomy in Male C_3 H Mice

	Pinealectomized	Sham-operated
Animals		
Number	8	9
Weight (g)	25.0 ± 0.7	24.4 ± 0.7
Organ weights (mg)		
Adrenals	3.2 ± 0.2	3.4 ± 0.1
Thyroid	2.5 ± 0.1	1.9 ± 0.03
Testicles	164.7 ± 5.6	145.3 ± 2.2
Pituitary	1.6 ± 0.2	1.2 ± 0.1

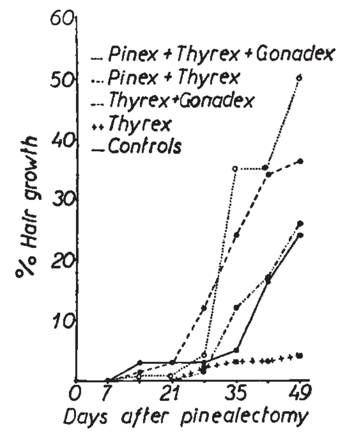

FIG. 2. Effect of pinealectomy upon hair waves in thyroidectomized and castrated-thyroidectomized mice.

Effects of pinealectomy in thyroidectomized and in castrated-thyroidectomized mice.—In Experiment 2, the hair growth was studied in the following 5 groups of mice: 1) castrated-thyroidectomized-pinealectomized, 2) castrated-thyroidectomized-sham operated, 3) thyroidectomized-pinealectomized, 4) thyroidectomized-sham operated and 5) sham operated controls.

The manner in which the clipped areas were covered by hair in the different groups is shown in Figure 2. The thyroidectomized mice were very slowly covered by hair. A larger area was covered in control mice and a slightly larger one in castrated-thyroidectomized mice. In the two groups in which pineal glands had been removed, the area covered by hair was much larger than in the other 3 groups, *i.e.*, the pineal gland removal accelerated the hair wave in the castrated-thyroidectomized mice. The effect is more clearly shown in the thyroidectomized mice where pinealectomy produced striking acceleration in the speed of the hair wave.

TABLE II

Effects of Pinealectomy in Castrated and Castrated-Thyroidectomized Mice

	Thyroidectomized		Castrated-thyroidectomized		Controls Sham-operation
	Pinealectomy	Sham-operation	Pinealectomy	Sham-operation	
Animals					
Number	13	8	12	6	6
Weight (g)	24.0 ± 0.6	24.0 ± 0.8	23.4 ± 0.5	23.0 ± 1.0	26.7 ± 0.7
Organ weights (mg)					
Adrenals	5.7 ± 0.1	6.0 ± 0.4	5.7 ± 0.1	6.0 ± 0.1	5.4 ± 0.4
Thyroid	—	—	—	—	2.8 ± 0.2
Testicles	171.5 ± 3.7	173.3 ± 5.0	—	—	150.9 ± 6.4

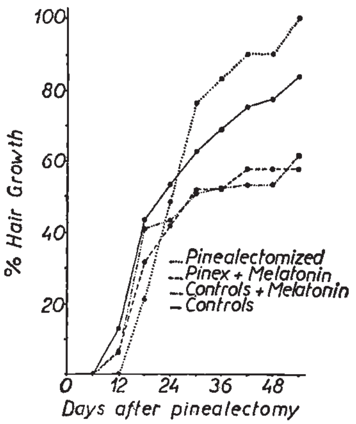

FIG. 3. Effects of daily administration of 100 μg of melatonin during 56 days in pinealectomized and sham operated mice.

The weight of the endocrine organs in these groups of animals is shown in Table II. An increase in the size of testicles was found in thyroidectomized ($p < 0.02$) and in thyroidectomized-pinealectomized mice ($p < 0.02$) as compared with the controls, but pinealectomy did not increase the size of the testicles in the thyroidectomized mice. No significant changes were found in the weight of the adrenals in any of the groups.

Effects of melatonin: Melatonin was administered during 56 days on a dose of 100 μg daily to pinealectomized and sham-operated mice. The following groups of mice were studied: 1) pinealectomized injected with melatonin, 2) pinealectomized non-injected, 3) sham-operated injected with melatonin and 4) sham-operated non-injected.

In Figure 3, the effects upon hair waves are presented. The clipped areas were covered by hair more slowly in the melatonin injected mice, either pinealectomized or sham-operated.

The weight of the organs is shown in Table III. There is thyroid hypertrophy ($p < 0.05$) in pinealectomized mice. The administration of melatonin decreased thyroid weight in pinealectomized mice ($p < 0.02$) but not in the sham-operated mice. Melatonin increased adrenal weight in the sham-operated mice ($p < 0.001$) but not in the pinealectomized mice.

DISCUSSION

The results presented in this paper indicate that the hair growth waves run faster in pinealectomized C_3H/Ep mice than in their sham-operated controls. This effect could be direct upon the skin or mediated through some endocrine alteration. If the thyroid hypertrophy found in the autopsy study indicated thyroid hyperfunction, acceleration of hair waves might be explained (6, 8, 10, 12, 13). The testicle hypertrophy would produce an opposite effect (9, 13, 15). The fact that no changes were found in the size of the adrenals does not prove that adrenal function was not modified.

The influence of the thyroid and gonads as mediators of the hair wave effect could be ruled out, as hair waves are also accelerated in the thyroidectomized and in the castrated-thyroidectomized mice. These data would suggest a direct effect of the pineal gland upon the run of the hair waves.

It is interesting to point out that the stimulation of the hair follicles after pinealectomy in normal mice is not so great as immediately after gonadectomy or adrenalectomy, where all the resting hair follicles start their anagen and produce a diffuse hair wave (9, 13, 15). On the other

TABLE III

Effects of Melatonin in Pinealectomized Mice

	Pinealectomized		Sham-operated	
	Melatonin	Non-injected	Melatonin	Non-injected
Animals				
Number	11	8	5	7
Weight (g)	25.6 ± 0.9	24.5 ± 1.0	26.4 ± 1.4	26.4 ± 1.4
Organ weights (mg)				
Adrenals	4.1 ± 0.7	4.2 ± 0.2	4.8 ± 0.1	3.5 ± 0.2
Thyroid	2.2 ± 0.01	3.0 ± 0.3	2.4 ± 0.3	2.1 ± 0.25
Testicles	139.2 ± 3.4	131.3 ± 10.0	140.0 ± 5.3	144.7 ± 3.3

hand, in thyroidectomized mice, pinealectomy produced a greater stimulation of the follicles than castration, as the hair waves in pinealectomized-thyroidectomized mice were faster than in castrated-thyroidectomized mice. Moreover, pinealectomy antagonized completely the effect of thyroidectomy, thus pinealectomized-thyroidectomized mice had very rapid hair waves, faster than those of control mice, in spite of the fact that thyroidectomized mice had very slow hair waves.

The pineal effect could be attributed to the secretion of melatonin as this substance slowed down the advance of the hair waves either in pinealectomized or sham operated mice.

The effects of melatonin on the pigmentation of the skin of amphibia are well known (16, 17), but this is the first time that the pineal gland and melatonin are shown to act upon the skin of mammals.

The hypertrophy of testicles after pinealectomy found in Experiment 1, but not in Experiment 3, has been obtained in rats by some investigators (19), and not by others (20). The time interval after the operation may have had an influence upon this difference (19). This hypertrophy was not found in thyroidectomized mice in Experiment 2.

The most constant endocrine alteration in our experiments was the marked thyroid hypertrophy found in pinealectomized mice. It agrees with the histological signs of thyroid hyperactivity found after pinealectomy in rats (21) and turtles (22), but unfortunately, no references on the weight of the thyroids are reported.

In Experiment 3, melatonin inhibited the thyroid hypertrophy in pinealectomized mice but did not alter the size of the thyroids in the sham-operated controls. There is a similar inhibitory effect by melatonin on the weight of the gland and the thyroid cell size, in rats treated with methylthiouracil (3) or with a chronic low iodine diet (24).

SUMMARY

The effect of the pineal gland on the run of the hair growth waves was studied in male C_3H/Ep mice.

Removal of the pineal gland produced acceleration of the hair waves. Hypertrophy of the thyroid and testicles was observed in the pinealectomized mice. The acceleration of the hair waves after pinealectomy was also seen in thyroidectomized and in castrated-thyroidectomized mice.

The daily administration of 100 μg of melatonin during 56 days slowed down the progression of the hair waves either in normal or in pinealectomized mice. Melatonin inhibited the thyroid hypertrophy found in pinealectomized mice, but did not have any effect upon the size of the thyroid in the sham-operated controls.

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